WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 3, 467-478 Research Article ISSN 2278 – 4357

DETECTION OF PESTICIDES IN BUFFALO MILK COLLECTED FROM DIFFERENT AREAS OF GANDHINAGAR AND AHMEDABAD BY THIN LAYER CHROMATOGRAPHY

Jivraj Makadiya[1] and Astha Pandey[1*]

[1]Assistant Professor, Institute of Forensic Science, Gujarat Forensic Sciences University, Gandhinagar-382007 Gujarat, India.

Article Received on
24 Dec. 2017,
Revised on 14 Jan. 2018,
Accepted on 04 Feb. 2018
DOI: 10.20959/wjpps20183-8566

***Corresponding Author**
Dr. Astha Pandey
Assistant Professor, Institute of Forensic Science, Gujarat Forensic Sciences University, Gandhinagar-382007 Gujarat, India.

ABSTRACT

Different pesticides have been used in tropical countries to control agricultural pests. Problem of the presence of pesticides in milk contributes to the serious issue of human health. The identification of organophosphorous (chlorpyrifos) pesticides in buffalo milk collected from different localities of Gandhinagar and Ahmedabad was performed. Milk was monitored by using TLC (thin layer chromatography) technique for 3 pesticides residues namely Dichlorovos, Chlorpyrifos and DDT (dichlorodiphenyl trichloroethane) to determine the degree of contamination. Extraction was performed with Hexane: Acetone: 1:1. Extracted milk samples spotted on TLC with pesticides standards. The analysis showed that most of the milk samples were contaminated by the pesticides. Identification of pesticides was done through RF (Retention factor) value and color of the spot developed after spraying the reagent with the standards. Confirmation of pesticides was done through GC-MS. Results showed that 40 % of the milk samples were contaminated with pesticides residues of organophosphorous (chlorpyrifos) was most significantly present in milk samples. The intake of the pesticide contaminated milk might pose health hazardous to humans in this locality.

KEYWORDS: Pesticides, Residues, TLC, Buffalo milk, Contamination, Gandhinagar and Ahmedabad.

INTRODUCTION

Pesticides are used worldwide to increase the production but many persistent residues of pesticides cause problem in environmental contamination and human health. India is the largest user of pesticides. Several pesticides contain the noxious substances that persist in environment for a long time.[1] Different types of organophosprous and organochlorine have been identified in milk sample. These pesticides can be absorbed by buffalo through their feed. Problem of the presence of pesticides in milk contributes to the serious issue of human health. Pesticides work to kill the pests or make them ineffective. In the same way, pesticides can affect the unintended individual, such as human. Pesticide residues have greater impact on human diet so contamination with these residues checked with greater concern in milk and milk products.[2,3]

To control the presence of pesticide residues in fresh milk and milk products is a big issue for producer, consumer and government due to the potential risk. Human milk as well as animal milk is contaminated through the contaminated food. These residues are too much persistent. They accumulate in body fat even in breast milk. These pesticide residues that move in human through milk samples cause different heart problems, endocrine disrupt and cancer. Applications of the regular pesticides firstly cause the acute effect then produce the chronic effect and may also cause death.[4,5,6]

Pesticides are being used to control the pests in the crops and to increase the quality of the crops. Intensive use of pesticides in agriculture as well as in the community health sector is the major cause of contamination of environment. Accumulation of these dangerous pesticides causes serious health issues in humans. Evidences show the presence of different organochlorine pesticides in crops, human fluids and also in meat. These pesticides also cause little sperm count, increased testicular cancer, different birth defects and different other reproductive defects. A broad range of pesticides are being used in world by farmers because of their wide spectrum activity and greater efficiency. Low cost is also another factor for their use. Because of the harmful effects of organochlorine pesticides these were banned in different time periods in different countries. The main problem of organochlorine pesticides was that they stick within the environment.[7,8]

India is basically an agricultural country. Livestock is playing a vital role in its economy. Buffalo is the major dairy animal of India and contributing maximum milk production. Pesticides may be classed into two classes' synthetic pesticides and the biological pesticides.

Wide spectrum pesticides can kill any species while selected pesticides only kill the selected species for which it has been made. Systemic pesticides are those which are absorbed by the plant. These pesticides come inside the plant circulation. DDT was very famous insecticide and in past its use was very common. 75 % pesticides are being in use by the developed countries. Pesticides save farmer money by killing the unwanted insects or pests in the crops by increasing production yield.[9,10]

Pesticides are causing disruptions in the endocrine glands. Hormones play vital role in normal development and pesticides disrupt hormones causing the abnormal development at early stages.

Organophosphate pesticides block the nerve impulses by reducing the acetyl cholinesterase activity. Most insecticides act on the nervous system of the insect's .Although there is remarkable difference between the nervous system of insects and the mammals but the toxicity mechanism is same in both Different tests are used to check the maximum residue limits of pesticides. Individual government and the international government set standards for the maximum residue limits. Environmental and the agricultural conditions affect maximum residue limits as these factors are not same in every country.[11,2]

In the modern agricultural practice, large numbers of pesticides are being used to protect plants from pests, weeds, fungi and insects. Indiscriminate and injudicious use of these pesticides has led to the accumulation of their residues in the environment in general and in foods of animal origin in particular. Persistent exposure to man and animals with the residues of pesticides results into a variety of health problems (Ritter, 1997).[12]

Synthetic pesticides are fat soluble, rapidly absorbed, stored in fatty tissues and slowly excreted (Hansen, 1987).[13]

The organophosphate pesticides are less persistent; but are highly toxic to higher mammals (Coulibaly and Smith, 1994).[14]

About 30 % of pesticides sold in the developing countries do not conform to the international quality standards thereby entailing potential risk to human, animal and the environment health. Biological magnification of pesticide residues in the food chain further compounds the problem (Gupta et al. 2000).[15]

Though organophosphate pesticides are less persistent and non-lipophilic but are highly toxic and their effects are irreversible (Coulibaly and Smith, 1994).[14]

In this monitory study milk samples collected from different animals were analyzed for the adulteration by different chemicals [16] and also for the presence of pesticides residues. The purpose of this study was to check whether milk was contaminated with pesticides or not. This study will be helpful for general public and farmers that they should use pesticides with caution.

MATERIALS AND METHODS

Sampling

All 30 milk sample were collected from different local Street vendor (public and educational institutions) & dairies from different areas of Gandhinagar & Ahmedabad and was preserved at -4°C Celsius temperature under refrigerator. The samples were collected in 100 ml screw capped sterilized plastic bottles. Formalin (formaldehyde) was used for preserved milk sample (0.4% HCHO added 2-3 drop/ 100 ml milk sample). All the possible precautions were taken to avoid external contamination at the time of collection of samples and during processing. Milk samples (n-30) of Samples were collected in clean, dry and neatly labelled sample containers and transported to laboratory in cold chain. After collection, all the samples were stored at -4°C until the extraction was done.

Pesticides Standards Collection

Pesticides standards of Chlorpyrifos, Dichlorovos and DDT were obtained from agricultural shop.

Sample Extraction

PROCEDURE -1

Milk sample is highest fatty, thicker and viscous sample so the pesticide extraction procedure is followed from research paper, they are as below. [16]

In 16 ml falcon-tube adds 4 ml milk + 4ml Hexane + 4 ml Acetone then centrifuge at 14000 Rpm for 10 min. At 29 °C Celsius temperature.

↓

Then this solution taken in test tube, upper layer removed.

↓

Then add 4 ml Hexane + 0.25 ml conc. Sulphuric acid.

↓

Upper layer of sample taken in another test tube & put on water bath for evaporated to dryness & then reconstitutes with Hexane, this solute used for spotting on TLC.

PROCEDURE -2[17]

Milk sample (10 ml) was transferred to a clean glass-stoppered cylinder and 80 ml of acetone: n-hexane (1:1, v/v) was added to it and shaken vigorously.

↓

The homogenate was allowed to stand until a clear separation of two layers had occurred. After the removal of the upper organic phase, the lower phase was re-extracted twice with 30 ml n-hexane.

↓

The precipitate was centrifuged at 4000 rpm and the remaining solvent was also combined.

↓

The concentrated n- hexane extract was taken in a 100 ml separating funnel and 35 ml concentrated sulphuric acid (sp. Gravity 1.84) was added to it drop wise. The mixture was allowed to stand until a clear separation of two layers.

↓

The upper clear phase was collected and lower phase of spent sulphuric acid was discarded. The collected upper phase was then washed with the distilled water.

↓

The collected extract was evaporated to dryness using a rotary evaporator and concentrated to about 5 ml with n-hexane.

↓

The final extracts were used for spotting on TLC plate.

Fig No. 1 Adulterated Milk Samples TLC

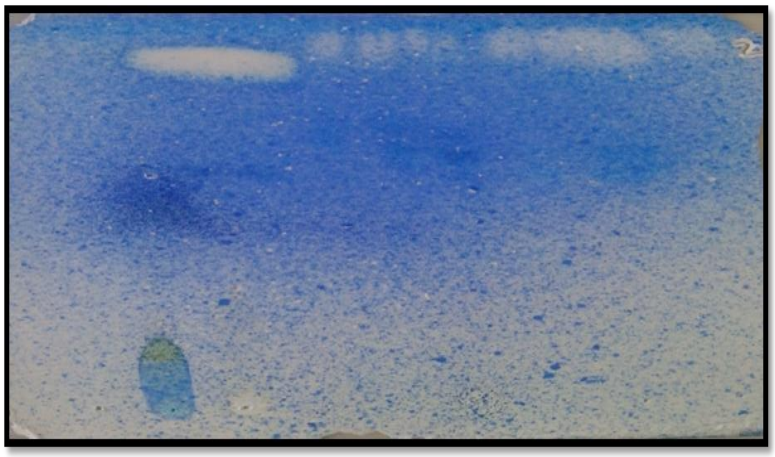

Fig No. 2 Adulterated Milk Samples TLC

Table No. 1 Adulterated Milk Samples Rf Value in Two Solvent System.

		Solvent Systems			
		Hexane: Acetone (7:3)		Hexane: Acetone (8:2)	
No.	**Spot name**	**Rf value**	**Color of the Spot**	**Rf**	**Color of the Spot**
1	DDVP control	0.24	Pale Yellow	**0.88**	Pale Yellow
2	DDT control	-		-	
3	Chlorpyrifos control	0.67	Pale Yellow	0.87	Pale Yellow
4	Milk extract-1	0.67	Pale Yellow	0.91	Pale Yellow
5	Milk extract-2	0.66	Pale Yellow	0.92	Pale Yellow
6	Milk extract-3	0.66	Pale Yellow	0.93	Pale Yellow
7	Milk extract-4	0.65	Pale Yellow	0.92	Pale Yellow
8	Milk extract-5	0.66	Pale Yellow	0.91	Pale Yellow
9	Milk extract-6	0.66	Pale Yellow	0.91	Pale Yellow
10	Milk extract-7	0.68	Pale Yellow	0.92	Pale Yellow
11	Milk extract-8	0.66	Pale Yellow	0.93	Pale Yellow
12	Milk extract-9	0.69	Pale Yellow	0.92	Pale Yellow

13	Milk extract-10	0.67	Pale Yellow	0.91	Pale Yellow
14	Milk extract-11	0.68	Pale Yellow	0.92	Pale Yellow
15	Milk extract-12	0.66	Pale Yellow	0.93	Pale Yellow
16	Milk extract-17	0.66	Pale Yellow	0.91	Pale Yellow
17	Milk extract-18	0.65	Pale Yellow	0.92	Pale Yellow
18	Milk extract-19	0.66	Pale Yellow	0.93	Pale Yellow
19	Milk extract-20	0.66	Pale Yellow	0.92	Pale Yellow
20	Milk extract-21	0.67	Pale Yellow	0.91	Pale Yellow

RESULTS

As per the observation of thin layer chromatography, after spraying with the zinc chloride and DPA (diphenylamine) reagent there was no immediate color development. The plate was then put in the oven for 10 min at 100 °C. Pale yellow spots developed after sometime which was for chlorpyrifos (Fig no. 1& 2) and out of 30 samples 12 samples showed the presence of chlorpyrifos. The control of DDVP (Fig No. 2) had shown deep bluish color whereas DDT control did not give positive test. The collected milk samples didn't show the presence of DDVP and DDT. Two solvent systems were used to see the better separation and it was found that Hexane and Acetone (8:2) showed better separation than Hexan: Acetone (7:3). (Table No. 1)

There were two spraying reagents which were used one the zinc chloride and diphenylamine and secondly the mercuric nitrate reagent. The former gave better results for chlorpyrifos which showed pale yellow color for the control and questioned milk samples whereas mercuric nitrate did not show any color for the control chlorpyriphos control of DDVP and the milk extracts. Zinc Chloride and Diphenylamine reagent also gave color for the DDVP control and not the milk samples indicating that the milk samples didn't contain any DDVP. The DDT control and milk samples showed negative with both the reagents indicating they were completely absent in the collected milk samples. The milk samples were found to be adulterated which were tested by color test[15]. The present study showed the adulteration of pesticides in the milk samples which could be further confirmed by quantitative techniques like HPTLC or GC which could not be performed in this study due to time constraint.

It could be concluded that OP pesticide residues (chlorpyrifos) were detected in twelve sample of buffalo's milk which could be due to their persistence in nature due to their slow decomposition rate, long half-life and high stability in the environment.

There are several reasons for the presence of pesticides in the milk samples. One reason being the fodder consumed by the cattles contain high content of pesticides. The water added to milk sample might increase the level of pesticides in milk.

CONCLUSION

Presence of pesticides in foods and especially in milk is a growing concern not only in India but also in other countries as they are widely consumed by all age group of people especially children. There has been several studies in India where different types of OP,OC and pyretheroids being detected in various food stuffs and milk samples. The present study was only to show the pesticides could also be one type of chemical adulterant present through different sources in the milk samples. It is only an eye opening study which states that milk samples might be contaminated with pesticides and we need to look further in the reduction level or enhance the quality control of milk sample in India. In this study 20 milk samples were collected from Buffalo. These milk samples were analyzed through TLC (thin layer chromatography) for the presence of pesticides residues (chlorpyrifos, DDT, Dichlorovos). The study findings revealed that 40 % of the collected milk samples were contaminated with the pesticides residues. The method used TLC can be used for the identification and separation of pesticides residues in milk samples. The findings of the study might help in extending awareness in dairy farmers and local people about pesticides and their hazardous effects on humans. Whereas the methodology might be useful to the analyst working in the particular field of milk analysis.

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